Epigenetic Influences on Oocyte Developmental Competence: Perifollicular Vascularity and Intrafollicular Oxygen

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Purpose: Studies indicating that the developmental competence of the human oocyte is influenced by the level of intrafollicular oxygen are described.

Methods: Perifollicular vascularity and dissolved oxygen content were determined by color Doppler ultrasonography and analysis of follicular fluid at ovum retrieval, respectively, in stimulated cycles for in vitro fertilization.

Results: Differences in the degree of perifollicular vascularity correlate with differences in the dissolved oxygen content of the corresponding follicular fluid. Oocytes with cytoplasmic and chromosomal disorders and embryos with multinucleated blastomeres and limited developmental ability were derived predominantly from underoxygenated follicles. Findings from several studies indicate that embryos with the highest implantation potential originate from follicles that are well-vascularized and oxygenated.

Conclusions: Follicular vascularity and oxygen content appear to be important determinants of oocyte competence. Possible causes of differences in follicle-specific vascularity and the potential effects of severe hypoxia on the normality of molecular and cellular processes during follicle growth and preovulatory development are discussed.

KEY WORDS: intrafollicular oxygen; perifollicular vascularity; oocyte competence.

INTRODUCTION

The relative contribution of extrinsic and intrinsic influences to the developmental competence of human embryos has been an important issue since the introduction of in vitro fertilization (IVF) and embryo culture as a routine clinical procedure nearly two decades ago. Efforts to improve the ability of human embryos

to develop in vitro have addressed one class of the extrinsic factors by attempting to create culture media and conditions that approximate some of the physical conditions and biochemistry thought to exist in the fallopian tube and uterus during the preimplantation stages. To this end, culture of early embryos in the presence of (i) cellular monolayers using widely different cell types of human and animal origin (1,2); (ii) peptide growth factors, cytokines (see Ref. 3 for review), serum proteins, and serum substitutes (see Ref. 4 for review); and (iii) changes in the composition and concentration of various salts and other ingredients such as antibiotics (5–7) have all, during the past decade, been reported to increase the frequency of development to the blastocyst stage. However, the conclusions that a particular medium or condition results in significantly improved embryonic development can be difficult to assess as many studies have been poorly designed or uncontrolled, and not infrequently, conflicting results have been reported (8). Increased frequencies of human preimplantation development observed in the presence of growth factors and cytokines, whose concentration in culture medium is generally empirically determined rather than based on known physiological levels, are particularly difficult to reconcile with the observation that for many of these factors, endogenous synthesis occurs or the corresponding cell surface receptors for these ligands are not detected (3). The notion that human embryos, like those of other mammals (4,9,10), can develop to the blastocyst in a comparatively simple culture medium has been a relatively recent “discovery,” and formulations that include only salts, amino acids, and a “defined” protein source such as albumin have been introduced in clinical IVF (5,6). However, it is necessary to consider that with media or conditions currently considered appropriate for human embryo culture, for some embryos within a typical cohort, development is not progressive and equivalent. Typically, some

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embryos arrest development during cleavage, while others that appear to develop to the blastocyst stage can, when examined carefully by high resolution light microscopy, be found to be growth retarded (11–13), or have an absent or numerically deficient inner cell mass (14), or both. Deficiencies in important biosynthetic activities indicative of reduced developmental potential have been described for blastocysts that appear grossly normal at the light microscope level (15–17). There is compelling evidence that the differential developmental competence exhibited by embryos in vitro is similar to the situation that prevails in vivo. A high proportion of embryos retrieved from the uterus of fertile women 5 days after intrauterine insemination was observed by Buster and his colleagues (18) to be fragmented, cleavage arrested, or developmentally abnormal.

Previously, pregnancy outcome data derived both from multicenter studies of large populations of fertile women, and women undergoing assisted reproduction procedures (intrauterine insemination, IVF, gamete intrafallopian transfer, etc.) have led to the conclusion that only a finite number of oocytes and embryos are developmentally viable in any natural or stimulated cycle (19–21). If this long-held notion is correct, then any improvements in culture systems may actually benefit only a subset of embryos (22), and additional advances in infertility treatment may require the identification of other extrinsic factors that influence oocyte and embryo developmental ability. In this respect, intrafollicular conditions to which the oocyte is exposed during preovulatory maturation may have an equivalent, if not more significant, role in determining developmental competence than the environment in which fertilization and embryo culture occur. Numerous animal and human studies have demonstrated that the developmental competence of the embryo is dependent largely on the normality of preovulatory oocyte maturation (see Ref. 23 for review). It has been recognized for many years that an association exists between chromosomal defects in the human oocyte and developmental failure at the embryonic and fetal stages. Numerical chromosomal defects that result from premature centromeric division, nondisjunction, and anaphase lag account for the majority of the disorders observed in oocytes and embryos. Cleavage-arrested human embryos with highly unusual chromosome numbers and distributions among blastomeres (termed catastrophic or chaotic mosaicisms) (24) may arise from oocytes in which assembly or organization of the metaphase spindles is defective (25,26). Although widely differing frequencies of aneuploidy have been

detected in oocytes from women undergoing ovarian stimulation for IVF (10–50%; see Ref. 23 for review), it seems that, on average, between 20 and 25% of normal-appearing metaphase II (MII)-stage oocytes may be chromosomally abnormal. The underlying frequency of aneuploidy appears to increase significantly with maternal age (>40y), in younger women whose oocytes exhibit specific cytoplasmic defects (>40%) (27), and for some women who repeatedly do not conceive after IVF, most, if not all, of the oocytes have been found to be aneuploid (28). The importance of these findings is the recognition of an intrinsic factor in human oocytes that can be a very significant determinant of embryo viability and that, for some women, may be the single most important determinant of their ability to conceive and maintain a normal pregnancy.

The extent to which aneuploidies detectable in mature human oocytes are a consequence of chromosomal defects that occur prior to the arrest of meiosis at the prophase I stage is unknown. It has been suggested that unrepaired DNA damage during early oogenesis could be a contributing factor in chromosomal malsegregations observed during the preovulatory resumption of meiosis (29). At present, it appears likely that the majority of chromosomal aneuploidies in human oocytes and some unknown fraction of chaotic mosaicisms in early embryos originate with the meiotic divisions initiated during preovulatory maturation and completed after sperm penetration. In support of this notion are cytogenetic results from several hundred karyotypes of MII-stage oocytes that had matured in vitro from the germinal vesicle stage (30,31). The findings indicate that aneuploidy is a relatively rare event when maturation occurs in vitro, with observed frequencies (<2%) significantly lower than those found in the in vivo matured oocytes of a similar population of fertile and infertile women (20–25%). Although the origin of in vitro (harvested after oophorectomy from small antral follicles in unstimulated ovaries) and in vivo (obtained by aspiration after ovarian stimulation) matured oocytes is different, the findings support two general conclusions for the human: (i) a predisposition to aneuploidy may not develop during early oogenesis or may not be a significant element in the generation of chromosomal abnormalities in the mature oocyte, and (ii) chromosomal malsegregations may not be an unavoidable consequence of meiotic maturation. These results suggest that follicle-specific influences may be detrimental to the normality of metaphase spindle organization and chromosomal segregation during the preovulatory meiotic divisions of the oocyte. While many studies have described quantitative and qualita-

tive differences in the protein, enzyme, hormone, growth factor, and cytokine composition of the fluids aspirated from follicles on the same and different ovaries, an identifiable biochemical pattern or profile that is clearly associated with the generation of chromosomally normal and chromosomally abnormal oocytes has not emerged. As discussed below, we and others have addressed the issue of follicular influences on the oocyte by asking whether specific physiological aspects of growth and maturation, such as the dissolved oxygen content of follicular fluid and the extent of perifollicular vasculature development, are determinants of oocyte and embryo developmental potential.

FOLLICULAR VASCULARITY AND OXYGEN CONTENT

Several recent studies indicate that expansion of the perifollicular vasculature during follicle growth and maturation is a determinant of the developmental competence of the corresponding oocyte. Nargund *et al.* (32) examined the relationship between ultrasound indices of follicular blood flow as measured by color Doppler imaging and pulsed Doppler spectral analysis, and oocyte recovery and subsequent production and morphological quality of preimplantation embryos. These investigators observed a statistically significant relationship between the extent of follicle-specific blood flow (measured as peak systolic velocity) immediately preceding aspiration and oocyte recovery and developmental competence after fertilization. Both within and between ovaries of the same patients, oocytes were obtained from follicles with no detectable flow at a lower frequency than from follicles that appeared to be well vascularized. Oocytes from poorly vascularized and apparently avascular follicles produced embryos that were morphologically inferior to those originating from their vascularized counterparts. Because outcome data were not reported, it is unknown whether the association between vascularity and embryo quality correlated with the variable developmental potential.

A positive correlation between the degree of follicular vascularity and outcome was described by Chui *et al.* (33) and Van Blerkom *et al.* (26). Based on the percentage of the follicular circumference in which vascularity could be detected by transvaginal power Doppler ultrasonography 2–3 hr before aspiration in patients undergoing ovarian stimulation for IVF, Chui *et al.* (33) classified 188 follicles greater than 16 mm in diameter into the following four groups: F1, <25%;

F2, 26–50%; F3, 51–75%; and F4, 76–100%. Although correlations with embryo morphology were not described, the results indicated that the developmental ability of the embryo after uterine transfer was related to the degree of vascularization of the follicle from which it originated. The frequency of triploidy was between 25 and 30% in oocytes derived from F1 and F2 follicles, in comparison to a frequency of <6% for oocytes that originated from F3 and F4 follicles. With respect to outcome, pregnancies occurred only in those patients whose embryos were derived from F3 and F4 follicles, with live births occurring only with embryos derived from F4 follicles.

The relationship among perifollicular vascularity, dissolved oxygen content of follicular fluid, and outcome after embryo transfer was examined by Van Blerkom *et al.* (26) for over 1000 individual follicles. Follicular blood flow characteristics were determined at the time an ovulatory dose of hCG was administered to women who had undergone the same protocol of ovarian stimulation for IVF. In this study, transvaginal color pulsed Doppler ultrasonography was used to measure perifollicular blood flow at different locations along the circumference of follicles greater than 18 mm in diameter. At ovum retrieval, the dissolved oxygen content of aspirated follicular fluids was measured from follicles whose ultrasonographic blood flow characteristics had been characterized previously. The stage of meiotic maturation, fertilizability, and progression through the early cleavage stages were determined empirically for each corresponding oocyte/embryo during in vitro culture and correlated with outcome when all of the embryos transferred were from follicles with known oxygen and blood flow parameters. The following three classes of follicles were identified according to the dissolved oxygen content of follicular fluid and the calculated resistive index (RI) of each follicle with measurable pulsatile flow: type A—RI, ≤ 0.5 ; dissolved oxygen content, $\geq 3\%$; and intense perifollicular coloration indicative of a well-vascularized follicle; type B—follicles with no perifollicular coloration detectable by color pulsed Doppler ultrasonography, an RI > 0.65 , and a dissolved oxygen content $< 3\%$; and type C—some detectable vascularization indicated by foci of perifollicular coloration, an RI between 0.45 and 0.60, and a dissolved oxygen content that varied between 2 and 3%. While these parameters did not show a significant association with the frequency of maturation (to MII) or fertilization, oocytes with severe cytoplasmic disorganization (27), two-cell embryos that contained at least one multinucleated blastomere, and embryos that arrested development

during the early cleavage stages were derived predominantly from type B follicles.

Three studies that used different analytical methods to assess the vascularity of human preovulatory follicles have come to the similar conclusion that follicular vascularity is associated with oocyte and embryo developmental potential. Similar to the observations of Nargund *et al.* (32) and Chui *et al.* (33), the findings of Van Blerkom *et al.* (26) demonstrated that, by routine ultrasonography, follicles of equivalent size and appearance, which often were adjacent to one and another, can exhibit very different levels of vascular development, blood flow characteristics, and dissolved oxygen contents. Preliminary results from an ongoing study indicate that the dissolved oxygen content and follicular blood flow characteristics are related to outcome after embryo transfer. In agreement with the findings of Chui *et al.* (33), we have observed that clinical pregnancy rates over 55% occur when all the embryos transferred on day 3 originated from type A follicles (34). To date, no clinical pregnancies have occurred in patients whose embryos were derived exclusively from type B follicles, and clinical pregnancy rates of approximately 30% have occurred with embryos that originated exclusively from type C follicles. It is important to emphasize that this study currently includes only those embryos that were assumed to be developing progressively in culture and appeared grossly normal at the level of the light microscope.

To the best of my knowledge, the notion that intrafollicular oxygen tension and oocyte chromosomal normality are related was suggested first by Gaulden (35), who proposed that hypoxic conditions could reduce levels of oocyte metabolism and alter the intracellular pH (pH_i). Gaulden postulated that a reduction in oocyte pH_i could lead to an instability in spindle microtubules or perturbations of microtubule assembly/disassembly dynamics, which in turn could contribute to chromosomal defects associated with anaphase lag and nondisjunction. She considered intrafollicular hypoxia to be a significant cause of the increased frequency of aneuploidy (e.g., trisomy 21) observed with advanced maternal age. The increase in perifollicular vascularity that appears to accompany follicular growth may be an effort to increase the availability of intrafollicular oxygen as the antrum expands. Presumably, the level of dissolved oxygen in follicular fluid is governed by the rate of diffusion across the follicular wall from the associated thecal vessels, as no thecal vessels have been shown to penetrate the growing follicle. Whether the percentage of dissolved oxygen in follicular fluids actually changes during the

follicular and preovulatory stages could not be determined by Van Blerkom *et al.* (26). However, Fisher *et al.* (36) measured the partial pressure of oxygen (pO_2) at aspiration in the follicular fluids of 20 women undergoing ovarian stimulation for IVF and concluded that pO_2 levels probably *declined* rather than increased in growing follicles. Oxygen tension in growing and preovulatory follicles was estimated by comparison with oxygen levels in blood taken from an arterialized well-perfused earlobe. These investigators concluded that an increase in ovarian blood flow during follicular maturation was not sufficient to accommodate the increased diffusion distance between the capillary network surrounding the follicle and its center, and as a consequence, intrafollicular levels of oxygen appear to decrease as the size of the follicle increases. While it is questionable whether oxygen tension determined from a well-perfused site is an accurate model to estimate intrafollicular pO_2 during growth, the occurrence of patient- and follicle-specific differences in perifollicular vascularity may not have been anticipated in this study. In support of this interpretation, an earlier study of follicular fluid pO_2 by Fraser *et al.* (37) observed marked differences in oxygen tension between similarly sized follicles (1–2 and >2 cm in diameter), which appeared to be patient specific. Measurements of pO_2 in human ovarian venous peripheral and arterial blood and in ovarian follicular fluids led to the conclusion that intrafollicular oxygen tension may be substantially lower than oxygen tension in peripheral and ovarian venous blood. A similar study by Shalgi *et al.* (38) found that the oxygen tension in follicular fluid differed over a ninefold range between follicles from the same and follicles from different patients. However, the pO_2 measured in most follicles was higher than measured in venous blood. While differences in methodology may explain the observed differences in intrafollicular and venous blood pO_2 values, these early studies show that the oxygen tension of preovulatory human ovarian follicles is patient and follicle specific and that preovulatory follicles can be relatively oxygen rich or virtually anoxic (38).

Gosden and Byatt-Smith (39) estimated the oxygen content of large preantral follicles by including measurements of ovine granulosa cell respiration in vitro in a mathematical model of radial diffusion of oxygen across the epithelium of ovarian follicles. These investigators concluded that most of the available oxygen is consumed by the outermost layer(s) of granulosa cells such that the follicle, in general, experiences a hypoxic environment, while the follicular core associated with the oocyte is virtually anoxic. According to

this model, the intrafollicular oxygen content in growing follicles should rise with antrum formation and an increased penetration of oxygen into the interior of the follicle should result from a diffusion coefficient for oxygen that will be greater in fluid than when the follicle was a completely cellular structure. The persistence of a severely hypoxic or virtually anoxic condition would appear to be associated with the degree to which perifollicular vascular development occurs during follicle growth and maturation.

Studies of the relationship between outcome after embryo transfer and follicular vascularity and oxygen content described above do not indicate whether intrafollicular oxygen tension may influence the developmental competence of the oocyte directly or indirectly. Zeilmaker *et al.* (40) examined the effect of oxygen on mouse oocyte maturation in vitro by culturing germinal vesicle (GV) stage oocytes in 0% O₂ for 2 hr, followed by culture in a 5% O₂ environment for an additional 14 hr. Culture under presumed anoxic conditions was associated with the absence of GV breakdown (GVB) in approximately 90% of the oocytes, while subsequent culture in a 5% O₂ atmosphere resulted in GVB and maturation to MII at a frequency only slightly below normal levels. These findings suggested that an abrupt change in oxygen tension may be a trigger for oocyte maturation. In our studies of mouse oocyte maturation in vitro, we have found it to be extremely difficult to obtain a completely oxygen-free culture medium simply by incubation of medium in a 0% atmosphere. In contrast to the 90% reduction in the frequency of GVB reported by Zeilmaker *et al.* (40), culture of denuded GV-stage mouse oocytes in very low-oxygen environments (measured at 1% or less) depressed the frequency of GVB and progression to MII by about 15% from control values of 91% (Van Blerkom, unpublished). This apparent discrepancy may be related more to the composition of the culture medium and conditions used for in vitro maturation than to whether the dissolved oxygen content of the medium was actually 0 or $\leq 1\%$. For the human, our studies found no significant association between the frequency of maturation in vivo and the dissolved oxygen content of the corresponding follicular fluid (26). Although dissolved oxygen contents of 1.5% or less were observed in approximately 35% of fully grown preovulatory follicles, the sensitivity of the measurements has not permitted an anoxic designation for any of the 1400 follicles examined to date. However, maturation of the human oocyte in a relatively low-intrafollicular oxygen environment was observed to be associated with high frequencies of developmentally significant cyto-

plasmic defects (27) and abnormalities in chromosomal alignment on the MII spindle (26). It remains to be determined whether the dissolved oxygen content of follicular fluid actually increases during follicular growth or changes with respect to increased perifollicular vascularity and blood flow detectable by ultrasonography.

Persistent oxygen starvation in growing follicles may adversely influence oocyte metabolism (39) such that ATP generation occurs at levels that are inadequate to support the morphodynamic processes required for preovulatory nuclear and cytoplasmic maturation. For example, GV-stage mouse oocytes failed to resume meiosis when cultured in the presence of potent uncouplers of mitochondrial oxidative phosphorylation and, after approximately 45 min of incubation, had a net ATP content that was approximately 10-fold lower than normal (41). When returned to standard medium, the ATP content rose rapidly but achieved a steady-state level that was $\leq 50\%$ of normal, which was maintained during subsequent maturation in vitro. Inhibitor-treated oocytes resumed meiosis and matured to MII at a frequency that was comparable to controls. The competence of uncoupler-exposed mouse oocytes was examined by IVF and embryo culture. Rates of fertilization and development through the early cleavage stages were comparable to controls, but development to the blastocyst was reduced drastically (88% for controls, 13% for embryos derived from treated oocytes). Van Blerkom *et al.* (41) suggested that meiotic maturation could occur over a relatively wide range of oocyte ATP contents but that the developmental consequences of a reduced capacity of the oocyte to generate ATP may not be evident until the preimplantation stages, when elevated levels of ATP production by maternally inherited mitochondria are required for embryogenesis to progress.

A similar relationship between oocyte ATP content and embryo developmental potential for the human was suggested by Van Blerkom *et al.* (41). Within patient-specific cohorts where a predetermined number of oocytes were inseminated and embryos replaced, the outcome after embryo transfer was related to the ATP content of residual uninseminated oocytes, unfertilized oocytes, and dispermic and severely fragmented embryos. A statistically significant correlation was found to exist between outcome after embryo transfer and ATP levels in the remaining gametes and embryos. These findings suggest that developmental ability during the preimplantation period may be associated with an embryo-specific capacity to generate ATP and that this capacity may be established in the oocyte. It is

unknown whether oocyte-specific rates of ATP generation and utilization are related to the intrafollicular oxygen levels. If an increased availability of oxygen is a proximate determinate of oocyte developmental competence, then perhaps the significant preovulatory expansion of the cumulus oophorus associated with the hydration of a hyaluronic acid-based extracellular matrix may be one mechanism by which such an increase is accomplished. In this respect, the notion that unexpanded human oocyte/cumulus-corona cell complexes are often associated with poor embryo developmental competence, but not with inhibition of meiotic maturation or fertilization (42), may be related to the persistence of a low-oxygen condition in the immediate vicinity of the oocyte that could result in intracytoplasmic conditions inconsistent with the organization of a normal spindle. Although highly speculative, it may be of interest to determine whether follicular fluid contains macromolecules capable of oxygen transport and penetration through the cellular layers and gel-like matrix that surrounds the oocyte.

The results discussed above indicate that if intrafollicular oxygen tension has a direct affect on the oocyte, its influence may first be developmentally relevant during preovulatory maturation. Alternatively, differential intrafollicular oxygen tension may influence the normality of granulosa cell function and under a low-oxygen condition, result in a biochemical or physiological environment that is inconsistent with developmental competence but not with the resumption of meiosis. For example, the level of steroid hormone production by granulosa cells is related to oxygen concentration. As discussed by Gosden and Byatt-Smith (39) in their consideration of the role of oxygen concentration in ovarian follicles, (i) both side-chain cleavage of cholesterol and aromatization of androgens to estrogen require molecular oxygen, and (ii) a gradient of activity of enzymes involved in metabolism and steroidogenesis that is observed in the granulosa layer of rat Graffian follicles (43) may be related to a similar oxygen gradient. It is unknown whether such an oxygen gradient exists in the human follicles or whether levels of steroid production and other granulosa cell biosynthetic activities are associated with oxygen content. In this respect, differences in follicular oxygen also may be a determinant of the relative number and "health" of the granulosa cells. A significant proportion of granulosa cells is apoptotic in human follicles that exhibit a diminished capacity to produce progesterone or have low levels specific growth factors and cytokines that inhibit apoptosis when present at higher concentrations (44–46). It is unknown whether persis-

tent underoxygenation during the growth and early luteal phases is associated with a high proportion of follicles cells entering programmed cell death and apoptosis pathways. However, if such a relationship can be established, it could begin to provide a biological basis for follicle-specific differences in biochemistry that may be related to perifollicular vascularity and oocyte developmental competence.

Whether intrafollicular oxygen levels influence oocyte developmental competence directly or indirectly, or both, will require additional investigation. However, some indications of developmentally significant affects of severe hypoxia on MII human oocytes have been observed. Van Blerkom *et al.* (26) reported that oocytes from type B follicles, which are poorly vascularized and have dissolved oxygen contents of 1.5% or less, often contain spindle defects that may be responsible for chromosomal malalignment and scattering. In contrast, such defects were relatively rare in oocytes from well-vascularized and comparatively well-oxygenated follicles. Although the etiology of spindle abnormalities is unresolved, there is preliminary evidence that oocytes from type B follicles have a pHi and metabolic level that is lower than that in their type A counterparts (47). As noted previously, fertilization and early cleavage do not appear to be adversely influenced in oocytes from type B or C follicles. However, the significantly higher frequencies of cleavage arrest and blastomere multinucleation observed in embryos derived from poorly vascularized follicles suggest metaphase spindle defects that develop in oocytes experiencing an underoxygenated environment may contribute to abnormalities in chromosome number and distribution after fertilization.

An increase in perifollicular vascularity would benefit both gaseous and macromolecular exchange across the follicle wall. It seems likely that the production of angiogenic factors by granulosa cells in the growing follicle, such as vascular endothelial growth factor (VEGF), is a primary mechanism to achieve this end. While VEGF concentrations in type A, B, and C follicles differ by at most two- to threefold [generally threefold higher in type A than in type B (26)], this growth factor may be only one of several that are up-regulated in normally developing follicles. However, VEGF may be a particularly relevant molecule for analysis of angiogenesis during follicle growth, preovulatory maturation, and corpus luteum formation (48) because its expression can be regulated by the level of steroids and oxygen (49,50). The assumed inability of some follicles to respond to intrafollicular VEGF or other putative angiogenic promoters could

indicate an existing perifollicular vasculature that is unresponsive or one that, while responsive, never "sees" angiogenic promoters produced within the underlying follicle. Histologic analysis of human ovarian follicles led Gauden (35) to suggest that a proximate cause of intrafollicular hypoxia, especially in older women, may be the occurrence of a perifollicular capillary bed that is too distant from the wall of a growing follicle either to oxygenate the follicle adequately or, possibly, to be acted upon by follicle-derived angiogenic factors. An inability of a follicle to develop an extensive vasculature may also limit the extent to which steroid hormones and other regulatory molecules produced by granulosa cells can exit the follicle. For example, leptin, a protein involved in fat metabolism (51) and reproduction (52,53), recently has been found to be produced by human granulosa cells (54,55). In stimulated ovaries, the level of serum leptin increases after the administration of an ovulatory dose of hCG, and this increase appears to correlate with the outcome after IVF (54). Whether serum and follicular leptin levels are related to intrafollicular oxygen content and perifollicular vascularization needs to be determined. However, it seems reasonable to propose that traffic into and out of the follicle of molecules that may regulate follicle development or hypothalamic/pituitary function or affect the reproductive tract in anticipation of ovulation and implantation may be severely compromised in inadequately vascularized follicles.

In summary, follicular vascularization appears to have a critical influence on the developmental competence of human oocytes and early embryos. The stimulated human ovary, in which the degree of perifollicular vascularization is follicle specific, provides an ideal system for the identification of regulatory molecules and mechanisms which may promote or retard this process. The importance of such investigations will be manifest in a better understanding of the etiology of chromosomal abnormalities in the female gamete. This research may also begin to explain why increased frequencies of aneuploidy occur with advancing maternal age and perhaps why most of the oocytes and embryos produced by some infertility patients are chromosomally abnormal. Clinical relevance will be demonstrated by an improved ability to select oocytes for insemination and embryos for transfer either during cleavage or after continued culture to the blastocyst stage in "optimized" medium. Ultimately, a better understanding of follicle-specific epigenetic influences on oocyte competence may lead to new clinical protocols for infertility treatment in which optimized systems for

in vitro oocyte maturation are developed in order to preclude exposure to potentially adverse intrafollicular influences that may occur during preovulatory maturation.

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